

# Working Group Meeting Report

## “The Interface between Thrombosis and Inflammation”

July 30, 2007

8:00 am – 4:00 pm

9112/9116 Rockledge II

Bethesda, MD 20892

### Working Group Members (alphabetically):

- Douglas Cines, MD (*Chair*)
- Jane Freeman, MD
- Jose Lopez, MD
- Rodger McEver, MD
- Peter Newman, PhD
- Guy Reed, MD
- Wolfram Ruf, MD
- Dudley Strickland, PhD
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### NHLBI Participants:

- Charles Peterson, MD
- Pankaj Ganguly, PhD
- Traci Mondoro, PhD
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### CDC Staff:

- Craig Hooper, PhD

### Objectives

To identify the critical research directions that are needed: 1) To achieve better understanding of the pathophysiological mechanisms leading to thrombosis in acute and chronic inflammation. 2) To identify regulatory pathways and develop new targeted therapeutic interventions for thrombotic events in inflammatory processes. 3) To develop new research methodology utilizing novel technologies for investigating the biology of the disease processes and to detect and quantify subclinical disease.

### Introduction

Constituents of the hemostatic system gain access to the vasculature itself during inflammation as a result of vasodilation, increased permeation, enhanced retention, transluminal signaling and ectopic expression. This may serve a relevant physiologic function. Fibrin may help protect vessels against specific infections and other threats through physical containment and by providing a provisional scaffolding to guide the migration of inflammatory cells. Its cleavage releases chemoattractants involved in host response and repair. Vascular function may be impaired when activation of the inflammatory and hemostatic system persists and resolution of fibrin deposition is protracted. Coagulant proteins may take on novel functions within the intermittently or persistently inflamed vasculature as result of environmental differences from the intravascular milieu including: different expression patterns of proteolytic enzymes that generate diverse bioactive fragments, altered balance in concentrations of coagulation proteins and their inhibitors, generation of bioactive proteolytic products from these inhibitors, activation/inactivation of shared or novel receptors and signal transduction pathways expressed by resident mesenchymal and infiltrating hematopoietic cells, and vascular sequelae of protracted host response to perceived danger signals including alterations in fibrin degradation and revascularization.

Proteins secreted from activated platelets (e.g. PF4), neutrophils (defensins) and other cell types are found within the vessel wall in settings such as atherosclerosis in which chronic inflammation is a component of a more complex biological process, but the protective or pathological functions and metabolism of these retained proteins at sites of inflammation are not well described. Contact factor proteins may assume greater importance in hemostasis. Enzymes such as thrombin, factor Xa and plasminogen activators may enhance smooth muscle cell growth and contractility, plasminogen activators/receptors modulate migration of inflammatory cells, PF4 may alter antigen presentation, defensin modulates vascular tone and angiogenesis, while activated protein C down-regulates certain inflammatory responses, among many possible examples. However, whether modulation of the hemostatic system within vessels can be used to regulate beneficial or injurious inflammatory responses to foreign or self-antigens has received limited study.

Most inflammatory conditions have a predilection for specific vascular beds and/or specific organs. The affected vessels differ in size, the hemodynamic forces they are subjected to, inter-cellular junctions, composition of the matrix and cell wall, as well as bidirectional interactions with the underlying parenchyma (e.g. the neurovascular unit, pulmonary-capillary bed, the glomerulus) that affect vascular function and that require further study. Greater understanding of the heterogeneity in the vascular response to inflammation might facilitate development of imaging techniques to identify affected areas and follow new approaches to cell-directed therapies that would enhance drug localization and safety.

A number of the research projects in the areas of inflammation or thrombosis recently scratched the surface identifying new pathways and regulatory mechanisms. Stimulation of the research efforts on the interface of thrombosis and inflammation may develop new key targets for therapeutic interventions for thrombotic events in inflammatory processes, based on the similarity and overlapping of the pathophysiological events of thrombosis and inflammation.

### **Recommendations (not prioritized):**

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1. **Achieve a better understanding of the interactions between leukocytes and platelets and endothelial cells.** The intercellular interface that emerges among these cells as a result of inflammation provides a unique compartment that allows transfer of both beneficial and potentially injurious locally generated bioactive molecules. This is a sequential, bidirectional communication from the circulating cells and the endothelium that, in turn, transmits signals to the subendothelium. The mechanisms for recruitment, activation and retention of platelets and leukocytes and sequelae of this acquisition on the behavior of endothelium and underlying tissue cells require more in-depth analysis. The identification of the key points controlling such communication may lead to novel pharmaceutical interventions in both thrombosis and inflammation.
2. **Investigate how the cellular component of hemostasis affects the immune system.** Much knowledge has accumulated to conclude that inflammation directly affects and induces thrombosis. However, despite the evidence of platelets affecting immune competent cells through direct contact and soluble factors, there is an incomplete understanding of paracrine and autocrine loops between cells and their association with vascular integrity during inflammation.
3. **Study phenotypic reprogramming of circulating and vascular cells by inflammatory mediators.** Hematopoietic and vascular precursor cells may be generated or primed to alter their transcription profile which they carry into the periphery for the remainder of their life.

Addressing these phenomena would lead to better understanding of such pathological conditions as atherosclerosis, myocardial infarction, obesity, and metabolic syndrome.

4. **Investigate the role of bioactive molecules released from platelets, leukocytes and endothelial cells on thrombosis and inflammation.** Control of granule formation, trafficking of proteins, and signaling pathways that lead to mediator release from platelets and leukocytes needs better definition. The alternative pathways (shed membrane, microparticles, protein, lipid, DNA, RNA, etc.) of information transfer of bioactive molecules should be studied.
5. **Study activation of platelets/leukocytes by novel stimulants (bacteria, viruses, endo/exotoxins, DNA) that may act through unique pathways.** Such activators are expected to identify different profile of mediators that are beneficial to the host at certain stages of inflammation and could be inhibited without affecting hemostasis. This could lead to the discovery of safer and more targeted antithrombotics that act by down-regulating specific inflammatory mediators.
6. **Define/identify the receptors, ligands and substrates that are induced by inflammation within the vessel wall as well as in the circulation and study the sequale of these changes on signal transduction.** Receptors modulate the function of other receptors. Inflammation may alter the level or composition of potential co-receptor partners and thereby influence their biological specificity. Receptor function may change due to alterations in receptor number, subcellular distribution and post-translational modification (e.g. oxidation) as a result of inflammation as well as alteration in ligand concentration and novel cell surface chaperones. Levels of the receptor expression are likely to change over time and differ between inflamed and healthy tissue and may impact favorably on reparative processes, but induce tissue injury when such changes are protracted and intense. Addressing this issue will benefit the development of novel biomarkers and targeted biomolecular approaches for therapy of vascular abnormalities that lead to thrombosis.
7. **Study coagulation proteins/receptors/pathways that have dual functions in coagulation and inflammation.** It is important to investigate how their specificity and potency may depend upon co-factors induced on or within the vessel wall at sites of inflammation as a result in changes in titer, permeability, inhibitor levels, ectopic protein expression, co-factors and changes in substrate preference and enzyme activity due to alterations within the intramural microenvironment.
8. **Further investigate the formation and modulation of the provisional matrix.** The composition of the provisional matrix is likely to be complex and has received insufficient study. Changes in composition and rates of formation and dissolution may affect both host defense and repair.
9. **Develop a core facility for aging of animals.** Such a facility would be of cross-institutional interest because many inflammatory, thrombotic, metabolic and degenerative diseases are age-dependent. It is not feasible for individual investigators to “age” diverse animal colonies, though a central facility could share these reagents with many investigators.
10. **Develop animal models that are informative for understanding the interactions between inflammation and thrombosis.** The mouse has the advantage of genetic manipulation but there will always be issues of relevance that will ultimately require analysis in human disease populations. However, the mouse can provide data for hypothesis building

and pilot intervention studies. Several complex models of inflammation have been developed (e.g. inflammatory arthritis, bowel disease, SLE, etc.) but their effect on the propensity for thrombosis remains to be studied. For many purposes, relevant changes may develop over shorter times permitting inflammation to be induced in wild type mice repetitively stimulated with antigen, cytokines, LPS, etc. or genetically engineered to express specific mediators of interest.

11. **Implement the Biologic Systems approaches.** The mouse represents a good model because it provides an opportunity to combine genetic approaches with the use of inhibitors of specific downstream pathways that would lead to the better understanding of the mechanistic issues. There is a need to develop longitudinal non-invasive, repetitive, dynamic imaging and in vivo biochemical sensing approaches to study initiation, propagation and resolution of chronic localized inflammatory and coagulant processes.
  12. **Develop novel techniques to visualize and monitor vascular processes in vivo.** Further improve laser microdissection, fixation, microarray, and other detection methods to identify alterations on and within inflamed vasculature. Develop and utilize new tagged antibodies in vivo for confirmation of differences between normal and inflamed tissue. Such approaches would permit both serial imaging and targeted therapeutics.
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